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ORIGINAL ARTICLE

The predictive role of sTWEAK levels in pregnant women with first-trimester vaginal bleeding*

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ABSTRACT

Purpose: To investigate the relationships of TNF-related weak inducer of apoptosis (sTWEAK), a cytokine related to the TNF superfamily, its newly described soluble receptor sCD163, and the sTWEAK/sCD163 ratio with perinatal outcomes in women with first-trimester vaginal bleeding.

Materials and methods: Seventy (41 threatened abortion and 29 control) gestational-age-matched (6–14 weeks) pregnant women were included in the study. Antenatal complications (gestational diabetes, preeclampsia, intrauterine growth restriction, oligohydramniosis, polyhydramniosis), and perinatal outcomes (delivery mode, birth weight, delivery week) were recorded. Women with vaginal bleeding were divided into subgroups by pregnancy outcome (miscarriage or live birth) and subchorionic hematoma incidence. Statistical analyses were performed using the Student's *t* test, Mann–Whitney *U* test, chi-square test, and Pearson's correlation coefficient. *p* Values $<.05$ were considered as statistically significant.

Results: There were no statistically significant differences in sTWEAK or sCD163 levels, in sTWEAK/sCD163 ratios, or antenatal complications between threatened abortion and control patients. Higher sTWEAK levels were significantly correlated with higher rates of miscarriage in the threatened abortion group ($p = .014$). sCD163 levels were significantly lower in the subchorionic hematoma subgroup of the threatened abortion group ($p = .043$).

Conclusions: sTWEAK levels may predict the risk of miscarriage in pregnant women with first-trimester vaginal bleeding.

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Vaginal bleeding; pregnancy; sTWEAK; sCD163; miscarriage

Introduction

Vaginal bleeding is a major pregnancy complication, with a rate of 7 – 25% in the first trimester [1]. Vaginal bleeding may be related to chromosomal or anatomical abnormality or infection in 50% of the cases, whereas the remaining 50% have no known etiology [2]. The most critical concern is the viability of the pregnancy during or after the bleeding. Although various therapeutic and preventive management strategies can be applied, the features predicting whether a complicated pregnancy will be terminated by miscarriage remain unclear.

Tumor necrosis factor (TNF)-related weak inducer of apoptosis (TWEAK), a cytokine related to the TNF superfamily, is located at chromosomal position 17p13.1 encoding a type 2 transmembrane protein [3]. TWEAK is expressed by monocytes, natural killer cells (NK), and dendritic cells of tissues or organs such as the heart, brain, pancreas, ovaries, placenta, and the immune system [3,4]. As demonstrated in mouse and human studies, TWEAK counteracts the cytotoxic and inflammatory functions of NK cells to prevent local cytotoxicity [5,6]. Soluble (s)TWEAK is one of the two forms that can be detected in serum or plasma [7]. sTWEAK may play a role in cellular proliferation, growth, migration, angiogenesis, and apoptosis by ligation to receptor Fn 14 [7,8]. CD163 is a newly described alternative soluble receptor similar to monocyte-macrophage surface receptors, and its expression may be induced by IL-13, TNFα, and sTWEAK [9]. sCD163 has been localized in the endometrial gland and surface epithelium of the uterus during the proliferative and luteal phases [5], and steroids have been demonstrated to regulate CD163 expression in sheep

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endometrium [10]. The TWEAK/CD163 interaction has been shown to play a role in inflammatory regulation *in vitro* and *in vivo* [11]. This TWEAK/CD163 axis is also suggested to act in cyclical hormonal changes during pregnancy and other physiological conditions [12]. According to experimental animal studies, CD163 may modulate ovarian steroid-related expression of IL-18 in sheep endometrial epithelial cells [10] and might affect the fate of pregnancy via the estrogen/progesterone-related decrease in IL-18 levels at the maternal–fetal interface [13]. IL-18 was also implicated in the remodeling of maternal circulation in human endometrium during implantation [14]. In light of these findings, we hypothesized that sTWEAK and CD163 levels may be related to pregnancy outcomes in patients with first-trimester vaginal bleeding. We investigated sTWEAK and CD163 levels, as well as the sTWEAK/sCD163 ratio.

Materials and methods

This study was approved by the Ethics Committee for Clinical Studies of the Mugla Sitki Kocman University Faculty of Medicine, and was performed in accordance with the guidelines of the Declaration of Helsinki. 41 patients with first trimester threatened abortion and 29 gestational age-matched controls were included in the study. The patients were recruited from the Department of Obstetrics and Gynecology at Mugla Sitki Kocman University Hospital and the Department of Obstetrics and Gynecology of İzmir Tepecik Training and Research Hospital between April and July 2015. Patients with multiple pregnancies, suspicion of fetal abnormalities, hypertension, diabetes, hematological disorders, uterine congenital abnormalities, or under anticoagulant therapy were excluded. All patients were between 6 and 14 gestational weeks. Antenatal complications (gestational diabetes, preeclampsia, intrauterine growth restriction, oligohydramniosis, polyhydramniosis) and perinatal outcomes (delivery mode, birth weight, gestational week at birth) were recorded in patients who reached term. After written informed consent was obtained from all subjects, venous blood samples were collected into vacutainer plastic tubes without additives or tubes containing EDTA. Blood samples were centrifuged at 1500 × *g* for 15 min, and the serum was separated and immediately stored at −70 °C until measurement of sTWEAK and sCD163.

Measurement of serum sTWEAK levels

Serum sTWEAK levels were measured with a human ELISA kit (sTWEAK için eBioscience, An Affymetrix Company, Austria) according to the manufacturer's instructions. sTWEAK levels were calculated from a standard curve and are expressed as pg/mL.

Measurement of serum sCD163 levels

Serum sCD163 levels were measured with a human ELISA kit (Human sCD163 ELISA Ready-SET Go! eBioscience Products) according to the manufacturer's instructions. The levels of sCD163 were calculated from a standard curve and are expressed as ng/mL.

Statistical analysis

All statistical analyses were carried out using SPSS 20.0 (SPSS, Inc., Chicago, IL) software. Distribution normality was evaluated by the Kolmogorov–Smirnov test. Normally distributed data are reported as mean ± standard deviation, and data that are not distributed normally are reported as median (minimum − maximum). Categorical variables are reported as frequency and percentage. Differences between groups were analyzed by Chi-square test. Comparisons of normally distributed groups were performed via Student's *t* test, and the Mann–Whitney *U* test was used for comparisons of data that were not normally distributed. Pearson's correlation coefficient was used to determine correlation. *p* Values $<.05$ were regarded as statistically significant.

Results

Mean age of the patients in the threatened abortion (TA) group was 29.4 ± 6.1 whereas 27.9 ± 4.8 in the control group. Median number of gravidity and parity in TA group was 3 and 1, respectively, whereas 1 and 0 in control group. Mean gestational age of the patients in TA group was 9.9 ± 2.6 and 9.4 ± 2.3 in the control group. Mean length of CRL was 40.2 ± 25.6 in TA group and 33.0 ± 21.1 in the control group. Gravidity, parity, and the levels of hemoglobin, APTT (activated partial thromboplastin time), PT (prothrombin time), and fibrinogen were statistically different among the groups (unshown data).

There were no statistically significant differences in perinatal outcomes among all recruited pregnant women, and no antenatal complications in women who reached term (excluding miscarriage), as shown in Table 1. There were also no statistical differences between groups by means of sTWEAK and CD163 levels and sTWEAK/CD163 ratio among groups as shown in Table 1.

Pregnant women with vaginal bleeding ($n = 41$) were further divided into miscarriage ($n = 11$) and live

Table 1. sTWEAK, CD163 levels and sTWEAK/CD163 ratio and perinatal outcomes of threatened abortion and control groups.

	Threatened abortion *n* (%)	Controls *n* (%)	*p* Value
sTWEAK (pg/mL)	512.4 (297.4–688.4)[c]	529.4 (324–2110.4)[c]	.346[d]
CD163 (ng/mL)	683.25 ± 331.4[a]	674.20 ± 250.8[a]	.902[b]
sTWEAK/CD163	0.9 ± 0.5[a]	0.9 ± 0.4[a]	.453[b]
Delivery mode			
Vaginal delivery	11 (26.8)	5 (17.2)	
Primary C/S	12 (29.3)	14 (48.3)	.007*
Previous C/S	7 (17.1)	10 (34.5)	
Miscarriage	11 (26.8)		
Antenatal complication (miscarriage cases excluded) ($n = 59$)			
Gestational diabetes mellitus	2 (6.7)	5 (17.2)	.254
Preeclampsia	2 (6.7)	4 (13.8)	.424
Intrauterine growth restriction	7 (23.3)	5 (17.2)	.748
Oligohydramniosis	3 (10.0)	4 (13.8)	.706
Polyhydramniosis	3 (10.0)	–	

CD: cluster of differentiation.
[a]Mean (±SD).
[b]Student's *t* test was used for statistical analysis.
[c]Median (min–max).
[d]Mann–Whitney *U* test was used for statistical analysis.
*The Chi-square test was used for statistical analysis.
$p < .05$ was considered statistically significant.
C/S: Cesarean section.

Table 2. sTWEAK and CD163 levels and sTWEAK/CD163 ratio in miscarriage and live birth subgroups of threatened abortion group.

	Miscarriage	Live birth	*p* Value
sTWEAK (pg/mL)	690.4 (368.4–1205.4)[a]	513.9 (297.4–854.4)	.014*[b]
CD163 (ng/mL)	660.7 ± 260.9c	691.5 ± 357.4	.797[d]
sTWEAK/CD163	1.2 ± 0.5	0.9 ± 0.4	.158

[a]Median (min–max).
[b]Mann–Whitney *U* test was used for statistical analysis.
[c]Mean (±SD).
[d]Student's *t* test was used for statistical analysis.
*$p < .05$ was considered as statistically significant.

birth subgroups ($n = 30$). sTWEAK levels in the live birth subgroup were significantly lower than in the miscarriage subgroup ($p = .014$), as indicated in Table 2.

The patients with threatened abortion were further divided according to subchorionic hematoma incidence, as shown in Table 3. sCD163 levels were significantly lower in the subchorionic hematoma subgroup ($p = .043$).

Positive correlations were found between sTWEAK levels and age ($p = .038$), gestational age ($p = .000$), and CRL (crown rump length) ($p = .001$), and also between the sTWEAK/sCD163 ratio and gestational age ($p = .045$), PT ($p = .041$), delivery week ($p = .0019$), and birth weight ($p = .012$) (unshown data).

Discussion

We analyzed the relationships of perinatal outcomes with sTWEAK and CD163 levels and sTWEAK/CD163 ratios in pregnant women with first-trimester vaginal bleeding, and concluded that the lower the sTWEAK level was, the lower the miscarriage risk became ($p = .014$). This study is the first to define a relationship between the fate of pregnancies with vaginal bleeding and sTWEAK levels, highlighting the need for further clinical and biochemical investigations.

Endogenous TWEAK levels have been investigated in an immunologically induced mouse model of miscarriage [15]. Despite an increase in TWEAK levels in the luminal epithelium and somewhat around the spiral arterioles at gestational day 9, and a decrease in Fn14 levels during this period, TWEAK levels were lower and FN14 levels higher than in the control group at the day of miscarriage (gestational day 9.5) in the intraperitoneally lipopolysaccharide-administered and immunologically induced experimental miscarriage study group [15]. This was the first study to suggest that changes in TWEAK/Fn14 levels might create an immunological imbalance at the fetal–maternal surface, potentially leading to pregnancy loss. In our study, sTWEAK levels were lower in the vaginal bleeding group than in healthy controls, but this difference was not statistically significant. In contrast to the findings in this immunologically induced experimental miscarriage study [15], we found that the sTWEAK levels were significantly higher in the miscarriage group than in the live birth group among the patients with vaginal bleeding. sCD163 levels have also been shown to increase in pregnancy compared to non-pregnancy [16]. Although we did not find a difference in CD163 concentration associated with vaginal bleeding, sCD163 levels were significantly lower in the subchorionic hematoma subgroup. These findings led us to contemplate different mechanisms in TWEAK and CD163 pathways for the development and continuation of pregnancy.

Table 3. sTWEAK and CD163 levels and sTWEAK/CD163 ratio in subchorionic hematoma subgroups of threatened abortion group.

	Subchorionic hematoma (+)	Subchorionic hematoma (−)	*p* Value
sTWEAK (pg/mL)	562.4 (297.4–854.4)[a]	533.2 (349.4–1205.4)	.969[b]
CD163 ng/mL (ng/mL)	565.6 ± 219.8[c]	775.3 ± 377.1	.043*[d]
sTWEAK/CD163	1.1 ± 0.5	0.9 ± 0.4	.157

[a]Median (min–max).
[b]Mann–Whitney *U* test was used for statistical analysis.
[c]Mean (±SD).
[d]Student's *t* test was used for statistical analysis.
*$p < .05$ was considered statistically significant.

Lédée et al. performed immune profiling using immune biomarkers in patients with recurrent implantation failure (RIF), and personalized the treatment protocols according to IL8/TWEAK levels, categorizing the patients as possessing higher or lower immune activation [17]. As endometrium is normally anti-adhesive and needs expression-specific chemokines or adhesion molecules from a mature immune system during embryonic attachment [18], the patients in the lower immune-activation group with diminished IL8/TWEAK levels were hypothesized to have impaired adhesion and angiogenesis mechanisms. Based on this hypothesis, the authors performed local damage in the mid-luteal phase, supported the luteal phase via hCG, and recommended sexual intercourse after embryo transfer in the lower immune-activation group, whereas estrogen and progesterone were used to support the luteal phase or prednisolone/intralipids were administered to control the proinflammatory environment in the immune-overactivation group. The researchers attained a 40% rate of live birth in 85.1% of patients [17]. Relating these results to our study, both the decrease of sTWEAK levels in patients with first trimester bleeding and the increase in sTWEAK concentration in the miscarriage subgroup might be regarded as feasible in such a dramatic immune dysregulation framework. Hence, different pathways may play roles in immune mechanisms in patients with only vaginal bleeding compared to those with bleeding and miscarriage.

In a recent study, TNFα production increased gradually toward the end of pregnancy, especially in fetal antimicrobial defense-related inflammatory reactions, rather than placental remodeling or growth [19]. Furthermore, a gradual increase in TNFα levels was suggested to demonstrate a limited inflammatory response and low resistance to pathogens in early gestation [19]. Similar to that study, we also detected correlations between sTWEAK levels and gestational week, CRL, and birth weight. Cytokine regulation was expected for normal pregnancy because excessive secretion of TNFα has been found to be related to implantation failure, pregnancy complications, and miscarriage in previous studies [20,21]. TWEAK was also previously found to act against the harmful effects of TNFα [22]. Based on these findings and the significant differences in sTWEAK levels between the miscarriage and live birth subgroups of pregnant women with vaginal bleeding in our study, we speculate that TWEAK may play an important role in spontaneous abortion risk, and it may be useful as a biomarker to predict miscarriage risk. Exploring biomarkers of miscarriage risk in symptomatic patients may be preferable to doing so in asymptomatic patients to reduce anxiety. This is supported by our results, as this biomarker was only significantly different in symptomatic patients with vaginal bleeding.

Our study offers a new perspective on the potential of sTWEAK as a biomarker for unfavorable pregnancy outcomes. Our results may play a preliminary role in creating nomograms for sTWEAK and CD163 levels and in improving preventive and therapeutic strategies in cases of spontaneous abortion risk.

Disclosure statement

The authors report no conflicts of interest.

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